

CLAIMS

What is claimed is:

1. An isolated and purified biologically active *piwi* family polypeptide comprising:
 - 5 (a) a polypeptide encoded by a nucleic acid sequence as set forth in any of SEQ ID NOs:1, 3 and 5;
 - (b) a polypeptide encoded by a nucleic acid having at least about 75% or greater homology to a DNA sequence as set forth in any of SEQ ID NOs:1, 3 and 5;
 - 10 (c) a polypeptide encoded by a nucleic acid capable of hybridizing under stringent conditions to a nucleic acid comprising a sequence or the complement of a sequence as set forth in any of SEQ ID NOs:1, 3 and 5;
 - (d) a polypeptide which is a biologically functional equivalent of a peptide as set forth in any of SEQ ID NOs:2, 4 and 6;
 - 15 (e) a polypeptide which is immunologically cross-reactive with antibodies which are immunologically reactive with peptides encoded by the nucleic acid sequences as set forth in any of SEQ ID NOs:2, 4 and 6;
 - 20 (f) a polypeptide encoded by a nucleic acid molecule capable of hybridizing to a nucleic acid molecule having the sequence of any of SEQ ID NOs:1, 3 and 5, or fragments or variants or complementary sequences thereof, under high stringency conditions; or
 - 25 (g) a polypeptide comprising a portion of a polypeptide of a), b), c), d), e), or f).
2. The polypeptide of claim 1, wherein the polypeptide comprises a vertebrate *piwi* family polypeptide.
3. The polypeptide of claim 2, wherein the polypeptide comprises a
30 human *piwi* family polypeptide.

4. The polypeptide of claim 1, wherein the *piwi* family comprises an amino acid sequence as set forth in any of SEQ ID NOs:2, 4 and 6.

5. The polypeptide of claim 1, modified to be in detectably labeled form.

5 6. An isolated and purified antibody capable of specifically binding to the polypeptide of claim 1.

7. The antibody of claim 6 which is a monoclonal antibody.

8. The antibody of claim 6 which is a polyclonal antibody.

9. A hybridoma cell line which produces the monoclonal antibody of
10 claim 8.

10. An isolated and purified antibody capable of neutralizing the biological activity of the polypeptide of claim 1.

11. The antibody of claim 10 which is a monoclonal antibody.

12. The antibody of claim 10 which is a polyclonal antibody.

13. A hybridoma cell line which produces the monoclonal antibody of
15 claim 11.

14. An isolated and purified polynucleic acid encoding a biologically active *piwi* family polypeptide of claim 1.

15. The polynucleic acid of claim 14, wherein the encoded
20 polypeptide comprises a vertebrate *piwi* family polypeptide.

16. The polynucleic acid of claim 15, wherein the encoded polypeptide comprises a mammalian *piwi* family polypeptide.

17. The polynucleic acid of claim 16, wherein the encoded polypeptide comprises a human *piwi* family polypeptide.

18. The polynucleic acid of claim 14, wherein the encoded
25 polypeptide comprises an amino acid sequence as set forth in any of SEQ ID NOs:2, 4 and 6.

19. The polynucleic acid of claim 18, further comprising a *piwi* family-encoding polynucleic acid sequence as set forth in any of SEQ ID NOs:1, 3 and 5.

5 20. The polynucleic acid of claim 14, further defined as a DNA segment.

21. The polynucleic acid of claim 14, further defined as positioned under the control of a promoter.

22. The polynucleic acid of claim 14, further defined as a recombinant vector.

10 23. The polynucleic acid of claim 22, wherein the vector is a recombinant expression vector.

24. The polynucleic acid of claim 22, further defined as a nucleic acid fragment of up to 50,000 basepairs in length.

15 25. The polynucleic acid of claim 24, further defined as comprising at least a 1,000 nucleotide long contiguous stretch of a polynucleic acid sequence as essentially set forth in any of SEQ ID NOs:1, 3 and 5.

26. A recombinant host cell comprising the polynucleic acid of claim 14.

20 27. The recombinant host cell of claim 26, wherein the host cell is a procaryotic cell.

28. The recombinant host cell of claim 26, wherein the host cell is a eukaryotic cell.

25 29. A method of preparing a *piwi* family polypeptide, comprising: transforming a cell with the polynucleic acid of claim 14 to produce a *piwi* family under conditions suitable for the expression of said polypeptide.

30. A method of detecting in a sample an RNA that encodes the *piwi* family polypeptide encoded by the nucleic acid of claim 14, said method comprising the steps of:

- (a) contacting said sample under hybridizing conditions with the polynucleic acid of claim 14 to form a duplex; and
- (b) detecting the presence of said duplex.

31. A method of producing an antibody immunoreactive with a *piwi*
5 family polypeptide, the method comprising steps of:

- (a) transfecting a recombinant host cell with the a polynucleic acid of claim 14, which encodes a *piwi* family polypeptide;
- (b) culturing the host cell under conditions sufficient for expression of the polypeptide;
- 10 (c) recovering the polypeptide; and
- (d) preparing the antibody to the polypeptide.

32. The method of claim 31, wherein the polypeptide comprises a polypeptide as essentially set forth in any of SEQ ID NOs:2, 4 and 6.

33. The method of claim 31, wherein the polynucleic acid comprises
15 a polynucleic acid sequence as essentially set forth in any of SEQ ID NOs:1, 3 and 5.

34. An antibody produced by the method of claim 31.

35. A method of detecting a *piwi* family polypeptide, the method comprising immunoreacting the polypeptide with an antibody prepared
20 according the method of claim 31 to form an antibody-polypeptide conjugate; and detecting the conjugate.

36. A method of detecting a messenger RNA transcript that encodes a *piwi* family polypeptide, the method comprising the steps of hybridizing the messenger RNA transcript with the polynucleic acid of claim 14 to form a
25 duplex; and detecting the duplex.

37. A method of detecting a DNA molecule that encodes a *piwi* family polypeptide, the method comprising the steps of hybridizing DNA molecules with the polynucleic acid of claim 14 to form a duplex; and detecting the duplex.

38. An assay kit for detecting the presence of a *piwi* family polypeptide in a biological sample, the kit comprising a first container containing a first antibody capable of immunoreacting with a *piwi* family polypeptide of claim 1, wherein the first antibody is present in an amount
5 sufficient to perform at least one assay.

39. The assay kit of claim 38, further comprising a second container containing a second antibody that immunoreacts with the first antibody.

40. The assay kit of claim 39, wherein the first antibody and the second antibody comprise monoclonal antibodies.

10 41. The assay kit of claim 39, wherein the first antibody is affixed to a solid support.

42. The assay kit of claim 39, wherein the first and second antibodies each comprise an indicator.

15 43. The assay kit of claim 42, wherein the indicator is a radioactive label, a fluorescent label or an enzyme.

44. An assay kit for detecting the presence, in biological samples, of a *piwi* family polypeptide, the kit comprising a first container that contains a polynucleic acid identical or complimentary to a segment of at least ten contiguous nucleotide bases of the polynucleic acid of claim 14.

20 45. An assay kit for detecting the presence, in a biological sample, of an antibody immunoreactive with a *piwi* family polypeptide, the kit comprising a first container containing a *piwi* family polypeptide of claim 1 that immunoreacts with the antibody, with the polypeptide present in an amount sufficient to perform at least one assay.

25 46. A method of screening candidate substances for an ability to modulate *piwi* family biological activity, the method comprising the steps of:

- (a) establishing replicate test and control samples that comprise a biologically active *piwi* family polypeptide;
- (b) administering a candidate substance to the test sample
30 but not the control sample;

(c) measuring *piwi* family biological activity in the test and the control samples; and

(d) determining that the candidate substance modulates *piwi* family biological activity if the *piwi* family biological activity measured for the test sample is greater or less than the *piwi* family biological activity measured for the control sample.

47. The method of claim 46, wherein the candidate substance is further characterized as a candidate polypeptide, and further comprising the step of purifying and isolating a gene encoding the candidate polypeptide.

48. The method of claim 46, wherein the replicate test and control samples further comprise a cell that expresses a biologically active vertebrate *piwi* family polypeptide.

49. A recombinant cell line suitable for use in the method of claim 46.

50. A method of modulating *piwi* family polypeptide activity in a subject, the method comprising the step of administering to the subject an effective amount of a substance capable of modulating the *piwi* family polypeptide activity in the subject, whereby modulation of the *piwi* family polypeptide activity is accomplished.

51. The method of claim 50, wherein the step of administering further comprises administering an effective amount of a substance that modulates expression of a *piwi* family-encoding polynucleic acid in the subject.

52. The method of claim 51, wherein the substance that modulates expression of a *piwi* family-encoding polynucleic acid comprises an antisense oligonucleotide.

53. The method of claim 50, where the substance that modulates the *piwi* family activity comprises an anti-*piwi* family antibody.

54. The method of claim 53, where the anti-*piwi* family antibody comprises a monoclonal antibody.

55. The method of claim 50, wherein the *piwi* family activity comprises modulating cell growth, proliferation, division and combinations thereof, and wherein the step of administering comprises administering to the subject an effective *piwi* family-modulating amount of a *piwi* family-modulating substance.

56. The method of claim 50, wherein the subject is a vertebrate.

57. The method of claim 56, wherein the vertebrate is a warm-blooded vertebrate.

58. A method of treating a subject suffering from a disorder associated with *piwi* family biological activity, the method comprising: administering to the subject an effective amount of a *piwi* family biological activity-modulating substance, whereby treatment of the disorder is accomplished.

59. The method of claim 58, wherein the step of administering further comprises administering an effective amount of a substance that modulates expression of a *piwi* family-encoding polynucleic acid in the subject.

60. The method of claim 59, wherein the substance that modulates expression of a *piwi* family-encoding polynucleic acid comprises an antisense oligonucleotide.

61. The method of claim 58, wherein the substance capable of modulating *piwi* family activity in the subject comprises an anti-*piwi* family antibody.

62. The method of claim 61, wherein the anti-*piwi* family antibody comprises a monoclonal antibody.

63. A method of treating a subject suffering from a disorder associated with *piwi*-mediated biological activity, the method comprising administering to the subject a therapeutic composition which comprises a biologically active *piwi* family polypeptide, whereby treatment of disorder associated with *piwi*-mediated biological activity in the subject is accomplished.

64. The method of claim 63, wherein the therapeutic composition comprises a biologically active *piwi* family polypeptide and a pharmaceutically acceptable carrier.

65. The method of claim 63, wherein the *piwi*-mediated biological activity comprises modulation of stem cell growth, proliferation, division and combinations thereof, modulation of primordial germ cell proliferation, and combinations thereof; and the *piwi* family polypeptide is administered in an effective amount.

65. A method for modulating *piwi*-mediated biological activity in a cell,
10 the method comprising:

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- (a) delivering to the cell an effective amount of a DNA molecule comprising a polynucleotide that encodes a biologically active *piwi* family polypeptide; and
 - (b) maintaining the cell under conditions sufficient for expression of said polypeptide.

66. The method of claim 65, wherein the polypeptide comprises a polypeptide as essentially set forth in any of SEQ ID NOs:2, 4 and 6.

67. The method of claim 66, wherein the polypeptide is encoded by a polynucleic acid as essentially set forth in any of SEQ ID NOs:1, 3 and 5.

20 68. A method of culturing a primitive cell, the method comprising:

- (a) providing a culture comprising a primitive cell; and
- (b) delivering to the primitive cell a *piwi* family gene product, whereby growth of the primitive cell in a culture is accomplished.

69. The method of claim 68, wherein the primitive cell is maintained
25 in the culture for a time sufficient to produce a sustained cell culture.

70. The method of claim 68, wherein the *piwi* family gene product is delivered to the primitive cell by delivering to the primitive cell an effective amount of a polynucleotide that encodes a biologically active *piwi* family

polypeptide; and maintaining the primitive cell under conditions sufficient for expression of the polypeptide.

71. The method of claim 68, wherein the *piwi* family gene product is delivered to the cell by administration of a biologically active *piwi* family polypeptide to the culture.

72. The method of claim 68, further comprising:

- (a) collecting primitive cells;
- (b) depositing the primitive cells in contact with a feeder matrix, the feeder matrix comprising a *piwi* family gene product; and
- (c) culturing the primitive cells on the feeder matrix in the presence of media for a time sufficient to produce a sustained primitive cell culture.

73. The method of claim 68, wherein the feeder matrix comprises a recombinant cell comprising a polynucleic acid encoding a biologically active *piwi* family polypeptide.

74. The method of claim 68, further comprising establishing a monolayer of cells in the feeder matrix.

75. A transgenic or chimeric non-human animal having incorporated into its genome a polynucleic acid encoding a biologically active *piwi* family polypeptide, the polynucleic acid being present in said genome in a copy number effective to confer expression in the animal of the *piwi* family polypeptide.

76. The transgenic or chimeric non-human animal of claim 75, wherein said polynucleic acid comprises a human *piwi* family-encoding segment.

77. The transgenic or chimeric non-human animal of claim 75, wherein the expression of the *piwi* family polypeptide is conferred in reproductive tissue of the animal.

78. The transgenic or chimeric non-human animal of claim 75, wherein the expression of the *piwi* family polypeptide confers an increased

number of germline stem cells or primordial germ cells in the animal as compared to a normal animal.

79. The transgenic or chimeric non-human animal of claim 75, further comprising an additional polynucleic acid encoding a predetermined biologically active polypeptide, the polynucleic acid being present in said genome in a copy number effective to confer expression in the animal of the polypeptide.

80. A method of altering the phenotype of an embryonic animal, the method comprising:

- (a) providing a recombinant primitive cell comprising a polynucleic acid encoding a biologically active *piwi* family polypeptide;
- (b) transfecting the recombinant primitive cell with a polynucleic acid encoding a predetermined biologically active polypeptide;
- (c) transferring the transfected primitive cells into an embryo to confer expression of the predetermined biologically active polypeptide animal of the polypeptide, whereby the phenotype of the embryo is altered.

81. The method of claim 80, wherein the animal is a bird, and the transfected primitive cell is transferred into an egg containing an embryonic bird.

82. The method of claim 80, wherein the altered phenotype comprises a change in protein expression, a change in growth rate, feed efficiency, a change in stem cell count, a change in primordial germ cell count, disease resistance, or a combination thereof.

83. The method of claim 80, wherein the embryonic animal is permitted to develop into a fetus or animal.

84. A method of recovering a protein from a transgenic or chimeric non-human animal, the method comprising:

- (a) providing a transgenic or chimeric non-human animal having incorporated into its genome a polynucleic acid encoding a biologically active *piwi* family polypeptide, the polynucleic acid

being present in said genome in a copy number effective to confer expression in the animal of the *piwi* family polypeptide; and

- (b) recovering the biologically active *piwi* family polypeptide from the animal provided in step (a).

85. The method of claim 84, wherein the transgenic or chimeric non-human animal provided in step (a) further comprises another polynucleic acid incorporated into its genome, the polynucleic acid encoding a predetermined biologically active polypeptide, the polynucleic acid being present in said genome in a copy number effective to confer expression in the animal of the predetermined polypeptide; and wherein the predetermined biologically active polypeptide is recovered in addition to or instead of the biologically active *piwi* family polypeptide.

85. The method of claim 84 or 85, wherein the transgenic or chimeric non-human animal provided in step (a) further comprises an avian, and the polypeptide is recovered from an egg produced by the avian.

86. A pharmaceutical composition comprising an isolated and purified biologically active *piwi* family polypeptide and a pharmaceutically acceptable carrier.

87. The pharmaceutical composition of claim 86, wherein the polypeptide comprises a polypeptide as essentially set forth in any of SEQ ID NOs:2, 4 and 6.

88. The pharmaceutical composition of claim 86, wherein the polypeptide is encoded by a polynucleic acid sequence as essentially set forth in any of SEQ ID NOs:1, 3 and 5.

89. The pharmaceutical composition of claim 86, further comprising a cell expressing the polypeptide.

90. A pharmaceutical composition comprising an isolated and purified polynucleic acid encoding a biologically active *piwi* family polypeptide and a pharmaceutically acceptable carrier.

91. The pharmaceutical composition of claim 90, wherein the encoded polypeptide comprises an amino acid sequence as essentially set forth in any of SEQ ID NOs:2, 4 and 6.

5 92. The pharmaceutical composition of claim 90, further comprising a *piwi* family-encoding polynucleic acid sequence as essentially set forth in any of SEQ ID NOs:1, 3 and 5.

93. The pharmaceutical composition of claim 90, wherein the polynucleic acid is a DNA segment.

10 94. The pharmaceutical composition of claim 90, wherein the polynucleic acid is positioned under the control of a promoter.

95. The pharmaceutical composition of claim 90, wherein the polynucleic acid further comprises a recombinant vector.

96. The pharmaceutical composition of claim 90, further comprising a cell expressing the encoded polypeptide.